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A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No. A-59553-2/DAV/JJD

Anticipated Classification of
this Application:

Class: Subclass:

Prior Application: 09/018,599

Examiner: NOT YET KNOWN

Art Unit: NOT YET KNOWN

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NUMBER EL465283783US

DATE OF DEPOSIT August 3, 2000

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SIGNED

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09/03/00
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Box PATENT APPLICATION FEE

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

This is a request for filing an

☐ Original

☒ Continuation

☐ Divisional

☐ Continuation-in-part

application under 37 C.F.R. 1.53(b), in the name of R. ANDREW CUTHBERTSON, for
OCULAR GENE THERAPY. This ☒ continuation ☐ divisional ☐ continuation-
in-part claims priority to pending application Serial No. 09/018,599, filed
on February 4, 1998, which is a continuation of application Serial No.
08/332,566, filed October 31, 1994.

1. (a) ☐ Enclosed is a new application.
- (b) ☐ Enclosed is a continuation-in-part application.
- (c) ☒ Enclosed is a copy of the prior application.
2. (a) ☐ Enclosed is a new Declaration.

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(b) X Enclosed is a copy of the prior executed Declaration as originally filed.

(c) Enclosed is a Combined Declaration/Power of Attorney.

3. (a) Enclosed is a Small Entity Affidavit.

(b) A Small Entity Affidavit is of record in the prior application.

4. X The filing fee is calculated below:

Claims as filed in the prior application, less any claims canceled by amendment below:

FOR:	(Col. 1) NO. FILED	(Col. 2) NO. EXTRA	SMALL ENTITY RATE	FEE	OR	OTHER THAN A SMALL ENTITY RATE	FEE
BASIC FEE				\$345	OR		\$690
TOTAL CLAIMS	<u>10</u> -20 = *	<u>0</u>	x 9 =	\$	OR	x 18 =	\$
INDEP CLAIMS	<u>1</u> -3 = *	<u>0</u>	x 39 =	\$	OR	x 78 =	\$
[] MULTIPLE DEPENDENT CLAIM PRESENTED			+130 =	\$	OR	+260 =	\$
*If the difference in Col. 1 is less than zero, enter "0" in Col. 2.			TOTAL	\$	OR TOTAL		\$690

5. X The Commissioner is hereby authorized to charge any additional fees which may be required, including extension fees, or credit any overpayment to Deposit Account No. 06-1300 (Our File No. A-59553-2/DAV/JJD).

6. X Our check in the amount of \$ 690.00 is enclosed.

7. Cancel in this application original claims of the prior application before calculating the filing fee. (At least one independent claim must be retained for filing purposes.)

8. X Amend the specification by inserting before the first line the sentence:

--This is a X continuation division continuation-in-part of pending application Serial No. 09/018,599, filed February 4, 1998, which is a continuation of application Serial No. 08/332,566, filed October 31, 1994, which issued as U.S. Patent No. 5,827,702 on October 27, 1998.--

9. (a) X Informal drawings are enclosed, 2 sheets.

(b) Formal drawings are enclosed, sheets.

10. (a) Priority of application Serial No. filed on in is claimed under 35 U.S.C. 119.

(b) The certified copy has been filed in prior application Serial No. filed on .

11. An Assignment is enclosed.

12. _____ The prior application is assigned of record to _____
GENENTECH, INC.

13. _____ A Power of Attorney by Assignee is enclosed.

14. X _____ The power of attorney in the prior application is to:

FLEHR, HOHBACH, TEST, ALBRITTON & HERBERT
(name)

Suite 3400, Four Embarcadero Center
(address)

San Francisco, California 94111-4187

(a) X _____ The power appears in the original papers in the prior application.

(b) _____ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.

(c) X _____ Address all future communications to:

DOLLY A. VANCE, ESQ.

FLEHR HOHBACH TEST ALBRITTON & HERBERT LLP

Suite 3400, Four Embarcadero Center

San Francisco, California 94111-4187

Telephone: (415) 781-1989

15. X _____ A preliminary amendment is enclosed. (Claims added by this amendment have been properly numbered consecutively beginning with the number next following the highest numbered original claim in the prior application.)

16. _____ A Prior Art Statement is enclosed.

17. X _____ I hereby verify that the attached papers are a true duplicate of prior application Serial No. 08/332,566 as originally filed on October 31, 1994.

Date: 8-3-00 Dolly
DOLLY A. VANCE, ESQ.
Reg. No. 39,054

Address of Signer: _____ X _____ Attorney or agent of record

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP
FOUR EMBARCADERO CENTER, SUITE 3400
SAN FRANCISCO, CA 94111-4187
TEL: (415) 781-1989

Filed under Section 1.34(a)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:)	Examiner: Not Yet Known
)	
R. Andrew Cuthbertson)	Group Art Unit: Not Yet Known
)	
Cont. of Serial No. 09/018,599)	
)	
Filed: August 3, 2000)	
)	
For: Ocular Gene Therapy)	
_____)	

CERTIFICATE OF MAILING

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Hammid Sanchez

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, DC 20231

Sir:

Please enter the following amendments prior to substantive review of
this case.

The Commissioner is hereby authorized to charge any additional fees
which may be required, including extension fees and terminal disclaimer fee, or
credit any overpayment to Deposit Account No. 06-1300 (Order No. A59553-
2/DAV/JJD).

In the Claims:

Please cancel Claims 1-12 and insert the following.

--1. A method of treating a genetic ocular disease comprising incorporating
exogenous nucleic acid into an *in situ* ocular cell under conditions permissive

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Filed: August 3, 2000

for the uptake of said exogenous nucleic acid, said exogenous nucleic acid encoding a protein associated with said ocular disease, whereby said exogenous nucleic acid is expressed.

2. The method of claim 1, and wherein said genetic ocular disease is autosomal retinitis pigmentosa.
3. The method of claim 1, and wherein said genetic ocular disease is autosomal dominant retinitis punctata albescens.
4. The method of claim 1, and wherein said genetic ocular disease is butterfly-shaped pigment dystrophy of the fovea.
5. The method of claim 1, and wherein said genetic ocular disease is adult vitelliform macular dystrophy.
6. The method of claim 1, and wherein said genetic ocular disease is Norrie's disease.
7. The method of claim 1, and wherein said genetic ocular disease is blue cone monochromasy.
8. The method of claim 1, and wherein said genetic ocular disease is choroideremia.
9. The method of claim 1, and wherein said genetic ocular disease is gyrate atrophy.
10. A method of treating an ocular disease comprising incorporating exogenous nucleic acid into an *in situ* ocular cell under conditions permissive for the uptake of said exogenous nucleic acid, said exogenous nucleic acid

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encoding a protein associated with said ocular disease, whereby said exogenous nucleic acid is expressed, wherein said disease is lysosomal storage disease.--.

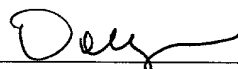
REMARKS

Claims 1 and 10 have been added as independent claims. Claims 2-9 have been added as claims depending from Claim 1. Support for these claims is found throughout the specification. Specifically, support for claims 1-9 is found at page 9, line 30 to page 10, line 6. Support for Claim 10 is found at page 10, lines 18-31.

Applicant believes that the claims are now in form for allowance. Applicant respectfully requests such a favorable finding and processing of the application to issuance. If there are any unresolved issues, the Examiner is requested to call the attorney signing below at (415) 781-1989.

Respectfully submitted,

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP



Dolly A. Vance
Reg. No. 39,054

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP
4 Embarcadero Center
Suite 3400
San Francisco, CA 94111
(415) 781-1989
Date: August 3, 2000

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APPENDIX

1. A method of treating a genetic ocular disease comprising incorporating exogenous nucleic acid into an *in situ* ocular cell under conditions permissive for the uptake of said exogenous nucleic acid, said exogenous nucleic acid encoding a protein associated with said ocular disease, whereby said exogenous nucleic acid is expressed.
2. The method of claim 1, and wherein said genetic ocular disease is autosomal retinitis pigmentosa.
3. The method of claim 1, and wherein said genetic ocular disease is autosomal dominant retinitis punctata albescens.
4. The method of claim 1, and wherein said genetic ocular disease is butterfly-shaped pigment dystrophy of the fovea.
5. The method of claim 1, and wherein said genetic ocular disease is adult vitelliform macular dystrophy.
6. The method of claim 1, and wherein said genetic ocular disease is Norrie's disease.
7. The method of claim 1, and wherein said genetic ocular disease is blue cone monochromasy.
8. The method of claim 1, and wherein said genetic ocular disease is choroideremia.
9. The method of claim 1, and wherein said genetic ocular disease is gyrate atrophy.
10. A method of treating an ocular disease comprising incorporating exogenous nucleic acid into an *in situ* ocular cell under conditions permissive for the uptake of said exogenous nucleic acid, said exogenous nucleic acid encoding a protein associated with said ocular disease, whereby said exogenous nucleic acid is expressed, wherein said disease is lysosomal storage disease.

OCULAR GENE THERAPY

FIELD OF THE INVENTION

The invention relates to methods of ocular gene therapy.

BACKGROUND OF THE INVENTION

5 Gene therapy treatments are rapidly becoming a reality, with several dozen gene therapy protocols approved by the National Institutes of Health, many of which being currently underway.

10 There are a number of ocular diseases and conditions which could be suitable for treatment with ocular gene therapy. These diseases fall into two categories, ocular disease caused by a specific genetic disorder, whether dominant or recessive, and diseases which have
15 no currently known genetic basis but instead could be treated with the introduction of genes expressing proteins useful in the treatment of the condition.

In the first category, there are a number of diseases for which the underlying genetic defect is known. Autosomal retinitis pigmentosa, both dominant and
20 recessive, may be caused by as many as 50 different mutations in the rhodopsin gene (Bok, Invest. Ophthalm. and Visual Sci. 34(3):473 (1993)). Autosomal dominant retinitis punctata albescens, butterfly-shaped pigment

dystrophy of the fovea, and adult vitelliform macular dystrophy, have been correlated to a mutation in the peripherin/RDS gene (Kajiwara et al., Nature Genetics 3: 208 (1993); Nichols et al., Nature Genetics 3:202 (1993); Wells et al., Nature Genetics 3:213 (1993)). Norrie's disease (Berger et al., Nature Genetics 1:199 (1992)), blue cone monochromasy (Nathans et al., Science 245:831 (1989)), and choroideremia (Cremers et al., Nature 347:674 (1990); Merry et al., Proc. Natl. Acad. Sci. USA 89:2135 (1992)) have all been shown to be caused by genetic mutations. The gene for gyrate atrophy involves more than 60 different mutations in the mitochondrial enzyme ornithine aminotransferase (Bok, supra).

In addition to the diseases for which specific genetic mutations are known to cause the phenotype, there are a number of diseases for which the specific genetic component is unknown. These diseases may have a genetic basis or may be caused by other factors resulting in changes in protein expression. For example, age-related macular degeneration is a significant ocular disease among older patients. Retinoblastoma, anterior and posterior uveitis, retinovascular diseases, cataracts, inherited corneal defects such as corneal dystrophies, retinal detachment and degeneration and atrophy of the iris fall into this category, as do retinal diseases which are secondary to glaucoma and diabetes, such as diabetic retinopathy.

Finally, there are a number of conditions which are not genetically based but are still significant ocular diseases. For example, viral infections such as Herpes Simplex Virus (HSV) or cytomegalovirus (CMV) infections frequently cause significant symptoms, and may cause

blindness. Retinal detachment, diabetic retinal disease, retinal vein thrombosis, retinal artery embolism, allergic conjunctivitis and other ocular allergic responses, dry eye, lysosomal storage diseases, glycogen storage diseases, disorders of collagen, disorders of glycosaminoglycans and proteoglycans, sphingolipidoses, mucopolipidoses, disorders of amino acid metabolism, dysthyroid eye diseases, anterior and posterior corneal dystrophies, retinal photoreceptor disorders, corneal ulceration and other ocular wounds such as those following surgery are also significant conditions which do not have a known genetic component.

Recent work has shown that the retinal degeneration phenotype of the *rd* mouse, which has served as a model for the study of human retinitis pigmentosa for over 30 years, may be rescued by the expression of a bovine cGMP phosphodiesterase β -subunit in transgenic *rd* mice (Lem et al., Proc. Natl. Acad. Sci. USA. 15:442 (1992)). Similarly, the retinal degeneration slow (*rds*) phenotype of the *rds* mouse may also be corrected by the creation of transgenic mice expressing the wild-type *rds* gene product, a 39 kD membrane-associated glycoprotein (Travis et al., Neuron, 9:113 (1992)). However, as pointed out by several commentators, transgenic techniques are not directly applicable to human therapy, due to the uncertainties of transgene insertion (Zack, Arch. Ophthalmol. 111:1477 (1993), Bok, supra).

Additionally, *in vitro* gene transfer using a retroviral vector has been done on cells deficient in β -glucuronidase, an enzyme deficiency which is inherited in an autosomal recessive manner. After transformation with the gene coding for the enzyme, the β -glucuronidase

deficient cells exhibited normal enzyme activity (Stramm et al., Exp. Eye Res 50:521-532 (1990)).

5 Recently, two in vivo protocols using adenoviral vectors have been reported. Bennett et al., Investigative Ophtahalmology and Visual Science, 1994, 35(5):2535; Li et al., Investigative Ophtahalmology and Visual Science, 1994, 35(5):2543.

10 Therefore, there is a need for in vivo and in situ ocular gene therapy. Accordingly, it is an object of the invention to provide methods for the generation of genetically-engineered ocular cells, and specifically, methods for the generation of genetically-engineered in situ ocular cells.

SUMMARY OF THE INVENTION

15 The present invention is based on the determination that in situ ocular cells may be genetically-engineered to produce exogeneous proteins.

20 In one aspect, the invention provides methods for generating genetically-engineered ocular cells. The method comprises contacting an ocular cell with exogeneous nucleic acid under conditions which are permissive for the uptake of the exogeneous nucleic acid into the ocular cell.

25 In a further aspect, the invention provides methods for introducing exogeneous nucleic acids to an in situ ocular cell. The method comprises contacting the ocular cell with suitable exogeneous nucleic acid under conditions permissive for the uptake of the exogeneous nucleic acid into the ocular cell.

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In one aspect, the invention provides a method for treating an ocular disease. The method comprises incorporating exogeneous nucleic acid into an in situ ocular cell, wherein the exogeneous nucleic acid encodes a protein associated with the ocular disease.

In an additional aspect, the invention provides methods for treating ocular disease. The method comprises incorporating exogeneous nucleic acid into an in situ ocular cell, wherein the exogeneous nucleic acid encodes a protein useful in the treatment of the ocular disease.

In a further aspect, the invention provides ocular cells containing exogeneous nucleic acid.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B depict the histochemical X-gal staining of frozen sections of corneal epithelium. The blue X-gal staining of Figure 1A shows gene delivery of the β -galactosidase marker to the basal epithelial cells on the corneal surface, after debridement as outlined in Example 1. Figure 1B is the control, showing the lack of staining. Both sections were counterstained with eosin.

Figures 2A and 2B depict the histochemical X-gal staining of frozen sections of corneal endothelium. The blue X-gal staining of Figure 1A shows gene delivery of the β -galactosidase marker to the endothelial cells on the corneal surface. Figure 2B is the control, showing the lack of staining. Both sections were counterstained with eosin.

DETAILED DESCRIPTION OF THE INVENTION

5 The invention is based on the discovery that exogeneous nucleic acid may be introduced into ocular cells, and in particular in situ ocular cells. Thus, methods are provided for generating a genetically-engineered *in situ* ocular cell. The method comprises contacting an ocular cell with exogeneous nucleic acid under conditions that allow the ocular cell to take up the exogeneous nucleic acid into said ocular cell and express it.

10 By the term "in situ ocular cell" or grammatical equivalents herein is meant an ocular cell contained within the eye, i.e. in vivo. Ocular cells include cells of the lens, the cornea (both endothelial, stromal and epithelial corneal cells), the iris, the retina,
15 choroid, sclera, ciliary body, vitreous body, ocular vasculature, canal of Schlemm, ocular muscle cells, optic nerve, and other ocular sensory, motor and autonomic nerves.

20 By the term "genetically-engineered" herein is meant a cell or tissue that has been subjected to recombinant DNA manipulations, such as the introduction of exogeneous nucleic acid, resulting in a cell or tissue that is in a form not ordinarily found in nature. For example, the cell contains exogeneous nucleic acid.
25 Generally, the exogeneous nucleic acid is made using recombinant DNA techniques. It is understood that once a genetically engineered cell or tissue is made, it may replicate non-recombinantly, i.e. using the in vivo cellular machinery of the host cell, but will still be
30 considered genetically-engineered for the purposes of the invention.

By the term "nucleic acid" or grammatical equivalents herein is meant either DNA or RNA, or molecules which contain both ribo- and deoxyribonucleotides. The nucleic acids include genomic DNA, cDNA and oligonucleotides including sense and anti-sense nucleic acids. The nucleic acid may be double stranded, single stranded, or contain portions of both double stranded or single stranded sequence.

By the term "exogeneous nucleic acid" or "foreign nucleic acid" or "recombinant nucleic acid" or grammatical equivalents herein is meant nucleic acid which encodes proteins not ordinarily made in appreciable or therapeutic amounts in ocular cells. Thus, exogeneous nucleic acid includes nucleic acid which is not ordinarily found in the genome of the ocular cell, such as heterologous nucleic acid from other organisms. Exogeneous nucleic acid also includes nucleic acid which is ordinarily found within the genome of the ocular cell, but is in a form which allows for the expression of proteins which are not ordinarily expressed in ocular cells in appreciable or therapeutic amounts. For example, while human neurotrophins such as nerve growth factor (NGF) and neurotrophin-3 (NT3) are encoded within the genome of human ocular cells, they are not ordinarily expressed in human ocular tissue in significant or therapeutic amounts. Thus, a human gene is exogeneous to a human ocular cell if it contains a promoter or is in some form which allows for the increased expression of the protein within the ocular cell. Thus the genetically engineered ocular cell may contain extra copies of a gene ordinarily found within its genome. Alternatively, the exogeneous nucleic acid may encode a variant or mutant form of a naturally-occurring protein.

It is understood that once an exogeneous nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, i.e. using the in vivo cellular machinery of the host cell rather than in vitro manipulations; however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered "exogeneous" or "recombinant" for the purposes of the invention.

In the preferred embodiment, the exogeneous nucleic acid encodes a protein to be expressed. That is, it is the protein which is used to treat the ocular disease. In an alternative embodiment, the exogeneous nucleic acid is an anti-sense nucleic acid, which will inhibit or modulate the expression of a protein. In this embodiment, the exogeneous nucleic acid need not be expressed. Thus, for example, ocular tumor cells may express undesirable proteins, and the methods of the present invention allow for the addition of anti-sense nucleic acids to regulate the expression of the undesirable proteins. Similarly, the expression of mutant forms of a protein may cause ocular disease. It is possible to incorporate both anti-sense nucleic acid to reduce the level of expression of the mutant endogeneous gene as well as nucleic acid encoding a correct copy of the gene.

In an additional embodiment, the exogeneous nucleic acid may encode a regulatory protein such as a transcription or translation regulatory protein. In this embodiment, the protein itself may not directly affect the ocular disease, but instead may cause the increase or decrease in the expression of another protein which affects the ocular disease.

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limited to, autosomal retinitis pigmentosa, autosomal dominant retinitis punctata albescens, butterfly-shaped pigment dystrophy of the fovea, adult vitelliform macular dystrophy, Norrie's disease, blue cone monochromasy, choroideremia and gyrate atrophy. These may also be referred to as genetic ocular diseases.

In other embodiments, the ocular disease may not be caused by a specific known genotype (although they may be shown in the future to have a genetic component). These ocular diseases include, but are not limited to, age-related macular degeneration, retinoblastoma, anterior and posterior uveitis, retinovascular diseases, cataracts, inherited corneal defects such as corneal dystrophies, retinal detachment and degeneration and atrophy of the iris, and retinal diseases which are secondary to glaucoma and diabetes, such as diabetic retinopathy.

In addition, the term ocular disease includes conditions which are not genetically based but still cause ocular disorders or disfunctions. These include, but are not limited to, viral infections such as Herpes Simplex Virus or cytomegalovirus (CMV) infections, allergic conjunctivitis and other ocular allergic responses, dry eye, lysosomal storage diseases, glycogen storage diseases, disorders of collagen, disorders of glycosaminoglycans and proteoglycans, sphingolipidoses, mucopolysaccharidoses, disorders of amino acid metabolism, dysthyroid eye diseases, anterior and posterior corneal dystrophies, retinal photoreceptor disorders, corneal ulceration and other ocular wounds such as those following surgery.

By the term "conditions permissive for the uptake of

exogeneous nucleic acid" herein is meant experimental conditions which allow the in situ ocular cell to take up, and be transformed with, the exogeneous nucleic acid.

5 The permissive conditions will depend on the form of the exogeneous nucleic acid. Thus, for example, when the exogeneous nucleic acid is in the form of an adenoviral, retroviral, or adenoassociated viral vector, the permissive conditions are those which allow viral
10 infection of the cell. Similarly, when the exogeneous nucleic acid is in the form of a plasmid, the permissive conditions allow the plasmid to enter the cell. Thus, the form of the exogeneous nucleic acid and the conditions which are permissive for its uptake are
15 correlated. These conditions are generally well known in the art.

In a preferred embodiment, the nucleic acid encodes a protein which is expressed. In some embodiments, the expression of the exogeneous nucleic acid is transient;
20 that is, the exogeneous protein is expressed for a limited time. In other embodiments, the expression is permanent. Thus for example, transient expression systems may be used when therapeutic proteins are to be delivered for a short period; for example, certain
25 exogeneous proteins are desirable after ocular surgery or wounding. Alternatively, for on-going or congenital conditions such as retinitis pigmentosa or glaucoma, permanent expression may be desired.

In some embodiments, the exogeneous nucleic acid is
30 incorporated into the genome of the target cell; for example, retroviral vectors described below integrate into the genome of the host cell. Generally this is

done when longer or permanent expression is desired. In other embodiments, the exogeneous nucleic acid does not incorporate into the genome of the target cell but rather exists autonomously in the cell; for example,
5 many such plasmids are known. This embodiment may be preferable when transient expression is desired.

Permissive conditions depend on the expression vector to be used, the amount of expression desired and the target cell. Generally, conditions which allow in vitro
10 uptake of exogeneous cells work for in vivo ocular cells. In some cases, the physical structural characteristics of the eye are taken into consideration.

For example, when the target cells are corneal epithelial cells, permissive conditions may include the
15 debridement, or scraping of the corneal epithelium, in order to denude the corneal surface down to a basal layer of epithelium. The exogeneous nucleic acid is then added, in a variety of ways as described below.

Permissive conditions are analyzed using well-known
20 techniques in the art. For example, the expression of exogeneous nucleic acid may be assayed by detecting the presence of mRNA, using Northern hybridization, or protein, using antibodies or biological function assays.

Specific conditions for the uptake of exogeneous nucleic
25 acid are well known in the art. They include, but are not limited to, retroviral infection, adenoviral infection, transformation with plasmids, transformation with liposomes containing exogeneous nucleic acid, biolistic nucleic acid delivery (i.e. loading the
30 nucleic acid onto gold or other metal particles and shooting or injecting into the cells), adenoassociated

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virus infection and Epstein-Barr virus infection. These may all be considered "expression vectors" for the purposes of the invention.

5 The expression vectors may be either extrachromosomal vectors or vectors which integrate into a host genome as outlined above. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the exogenous nucleic acid. "Operably linked" in this context means that the
10 transcriptional and translational regulatory DNA is positioned relative to the coding sequence of the exogenous protein in such a manner that transcription is initiated. Generally, this will mean that the promoter and transcriptional initiation or start sequences are positioned 5' to the exogenous protein
15 coding region. The transcriptional and translational regulatory nucleic acid will generally be appropriate to the ocular host cell used to express the exogenous protein; for example, transcriptional and translational regulatory nucleic acid sequences from mammalian cells,
20 and particularly humans, are preferably used to express the exogenous protein in mammals and humans. Preferred are ocular cell transcriptional and translational regulatory sequences. Numerous types of appropriate
25 expression vectors, and suitable regulatory sequences are known in the art.

In general, the transcriptional and translational regulatory sequences may include, but are not limited to, promoter sequences, ribosomal binding sites,
30 transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. In a preferred embodiment, the regulatory

sequences include a promoter and transcriptional start and stop sequences.

5 Promoter sequences encode either constitutive or inducible promoters. The promoters may be either naturally occurring promoters or hybrid promoters. Hybrid promoters, which combine elements of more than one promoter, are also known in the art, and are useful in the present invention.

10 In addition, the expression vector may comprise additional elements. For example, for integrating expression vectors, the expression vector contains at least one sequence homologous to the host cell genome, and preferably two homologous sequences which flank the expression construct. The integrating vector may be
15 directed to a specific locus in the host cell by selecting the appropriate homologous sequence for inclusion in the vector. Constructs for integrating vectors are well known in the art.

20 Suitable retroviral vectors include LNL6, LXS_N, and LNCX.

25 Suitable adenoviral vectors include modifications of human adenoviruses such as Ad2 or Ad5, wherein genetic elements necessary for the virus to replicate in vivo have been removed; e.g. the E1 region, and an expression cassette coding for the exogenous gene of interest inserted into the adenoviral genome (for example Ad_{CV}CFTR₁₀).

30 By "protein associated with an ocular disease" herein is meant a protein encoded by a gene identified as the origin of a genetic ocular disease. Thus, for those

ocular diseases for which the genetic component has been identified, such as retinitis pigmentosa, the associated protein is rhodopsin. Thus, the absence or presence of the associated protein is either the result or cause of the genetic ocular disease.

By "protein useful in the treatment of an ocular disease" herein is meant a protein which is effective to alleviate the symptoms of the ocular disease. The ocular disease may be genetic, or may not have a genetic component. Thus, for example, ocular wounds, allergies, viral infections, ulcerations, etc. may be treated with useful proteins. For instance, gD is a protein useful in the treatment of herpes simplex virus infections, transforming growth factor β (TGFB) in corneal epithelial wounds; anti-IgE antibody for ocular allergy, and brain derived neurotrophic factor (BDNF) for retinal degeneration. Neural growth factor (NGF) and neurotrophin 5 (NT5), as well as fusions and/or mutants of these, may be used for retinal degeneration or to delay or prevent damage after retinovasular disease, or retinal detachment or glaucoma. These neurotrophic factors may also be used to treat optic nerve compression, trauma or demyelination. Immunosuppressive proteins may be used to treat graft rejection after corneal transplantation. Vascular endothelial cell growth factor (VEGF) antagonists, such as antibodies or small molecules, may be used to treat neovascular disorders of the retina and vitreous. Basic fibroblast growth factor has been shown to prolong photoreceptor life in rats (Faktorovich et al., Nature 347:83-86 (1990)).

In one embodiment, the exogeneous nucleic acid is delivered to corneal epithelial cells. Corneal

epithelial cells are subject to injury, allergic reactions and infections, among others. Thus proteins which are useful in the treatment of these conditions, and others, may be delivered via the present invention.

5 In another embodiment, the exogeneous nucleic acid is delivered to corneal endothelial cells. This is particularly significant since dysfunction of the corneal endothelial cells causes blindness. This layer is often damaged during cataract extraction, which is
10 currently the most common surgical operation in the U.S. In addition, since the corneal endothelium cannot regenerate, since cell division does not occur, the expression of proteins which cause division or regeneration of corneal endothelial cells could be a
15 significant treatment of corneal endothelial damage.

In another embodiment, exogeneous nucleic acid is introduced into the cells of the trabecular meshwork, beneath the periphery of the cornea. The trabecular meshwork is the outflow tract from the anterior chamber
20 of the eye, which allows aqueous humor (the fluid contained within the eye) to drain from the eye. This is significant since glaucoma is a common cause of visual loss in the U.S., and is a result of increased intraocular pressure. Therefore, the methods of the
25 present invention may be useful to regulate the outflow of aqueous humor and treat or cure glaucoma.

In one embodiment, the exogeneous nucleic acid is introduced to cells of the choroid layer of the eye. The choroid layer of the eye is part of the blood supply
30 to the retina, and thus may supply proteins to the retina. For example, BDNF (brain-derived neurotrophic

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factor) may be delivered in this manner to treat retinal degeneration.

5 In alternative embodiments, the exogeneous nucleic acid is introduced to cells of the retina, sclera or ciliary body. This last may be done, for example, for controlling production of aqueous fluid in the treatment or prevention of glaucoma.

10 Similarly, additional embodiments utilize the introduction of exogeneous nucleic acid to the cells of the retinal or ocular vasculature, cells of the vitreous body or cells of the lens, for example the lens epithelium.

15 "Animal" as used herein includes both humans and other animals and organisms. Thus the methods are applicable to both human therapy and veterinary applications. For example, the veterinary applications include, but are not limited to, canine, bovine, feline, porcine, equine, and ovine animals, as well as other domesticated animals including reptiles, birds, rabbits, and rodents such as
20 rats, mice, guinea pigs and hamsters. Valuable non-domesticated animals, such as zoo animals, may also be treated. In the preferred embodiment the animal is a mammal, and in the most preferred embodiment the animal is human.

25 In addition, the methods outlined in the present invention are useful in the creation of ocular disease animal models. That is, mutated copies of genes may be introduced into animals to create models for drug screening and therapy. For example, genes in which
30 mutations are known to cause ocular disease, or to play a role in ocular disorders, may be introduced into

animals such as rodents and rabbits, resulting in ocular disease. For example, granulocyte macrophage colony stimulating factor (GM-CSF) may be expressed in intraocular macrophages to create a retinal degeneration model. Similarly, S-antigen may be expressed in the eye to generate a model of uveitis.

The following examples serve to more fully describe the manner of using the above-described invention, as well as to set forth the best modes contemplated for carrying out various aspects of the invention. It is understood that these examples in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes. The references cited herein are expressly incorporated by reference.

15

EXAMPLES

Example 1

Delivery of Exogeneous Nucleic Acid to Corneal Epithelial Cells

A β -galactosidase-expressing recombinant adenoviral vector was constructed by first building a shuttle plasmid, in which the CMV/ β galactosidase expression cassette was flanked by adenovirus DNA sequences, such that when cotransfected with the adenoviral deletion mutant DNA dl324 into 293 cells, a homologous recombination event placed the β -galactosidase expression cassette into the E1 region of the virus. The resulting E1-deficient recombinant virus was thus replication incompetent in all cells but 293 cells, and expressed the E. coli β -galactosidase gene. This gene product may be assayed for its enzyme function, or assayed histochemically by exposing the enzyme to a

substrate, X-gal, resulting in a characteristic blue stain in the cells that are expressing the marker gene.

The recombinant, replication-incompetent viral vectors were produced in 293 cells, a human fetal kidney cell line, which expressed the E1 protein in trans.

20 μ of the 1×10^9 pfu/ml viral vector preparation was applied topically in a balanced, buffered salt solution (10 mM Tris, pH 7.5, 1 mM MgCl₂, 10% glycerol) to the intact corneal epithelium of anesthetized Sprague - Dawley rats. The viral solution was left in place for 30 minutes, and then the solution was removed and the eye surface coated with a protective aqueous ointment (Rugby Artificial Tears: white petrolatum, mineral oil and anhydrous lanolin). The animals were allowed to recover, and were then sacrificed 24 hours later. The eyes were frozen in Tissue Tek, histologic sections were cut at 7 μ m thickness, and the sections stained histochemically to detect β -galactosidase expression. Control animals received the vehicle alone topically, and their eyes were processed in the same way. Neither the test animals, nor the control animals showed β -galactosidase expression in their corneal epithelial cells.

The corneal epithelium is a stratified, squamous epithelium. The outermost cells are flattened and regularly physiologically slough from the surface, while being renewed from the less-well-differentiated cells below. We reasoned that perhaps because of the structure of the corneal epithelium, the outer epithelial cells, which were the cells to which we might have achieved gene transfer, had sloughed off by the time we examined the eyes 24 hours after gene delivery.

We reasoned that surgically removing the outermost layers of the cornea epithelium, prior to topical gene delivery, would allow gene delivery to these deeper, more long-lived cells, which might then express the delivered gene and survive in vivo for a prolonged period. Such a surgical removal of superficial epithelial cells is called 'debridement', and is done in conjunction with treatment for a number of cornea epithelial disease states, including corneal ulceration.

We therefore did an experiment to gauge the rate of recovery of the corneal epithelium in rats following superficial epithelial debridement. The cornea epithelium of anesthetized rats were scraped with a scalpel blade in a reproducible fashion. The eyes were then examined histologically at time intervals of 5 minutes, 1 hour, 16 hours and 2 days, to look for epithelial repair. We found that by 16 hours after the surgery, it was impossible to see a difference between an unoperated eye and an eye that had the superficial corneal epithelium removed.

Therefore, in a second series of experiments, we debrided the superficial corneal epithelium using the scraping procedure outlined above, and immediately applied $20\mu\text{l}$ of a 1×10^9 pfu/ml solution of recombinant adenovirus to the corneal surface for 30 minutes. Animals were then sacrificed 24 hours later as before. As shown in figures 1A and 1B, histochemical staining of sections of these eyes revealed extensive β -galactosidase marker gene expression in the corneal epithelial cells of the animals given topical adenoviral vector, while there was no expression in the control animals.

Example 2

Delivery of Exogenous Nucleic Acid to Corneal
Endothelial Cells

20 μ l of aqueous humor from the anterior chamber of
5 anesthetized rats was removed using a 30g needle and a
Hamilton syringe. This fluid was replaced with 20 μ l of
a 1×10^9 pfu/ml solution of the replication-incompetent
recombinant adenoviral vector described above which
delivered a β -galactosidase marker gene. Control rats
10 were treated in the same way, but received vehicle
alone. 24 hours later the animals were sacrificed, and
the eyes were mounted in Tissue Tek, frozen, sectioned
and stained for β -galactosidase protein. As shown in
Figures 2A and 2B, there was clear positive staining of
15 the majority of cells lining the posterior surface of
the cornea (the corneal endothelial cells) in the
animals that received the viral vector, but no staining
in the animals that received vehicle alone. Furthermore,
there was also staining of the cells of the ciliary body
20 epithelium in animals that received vector.

Example 3

Delivery of Exogenous Nucleic Acid to Choroid
Ocular Cells

5 μ l of a 1×10^9 pfu/ml solution of a β -galactosidase
25 expressing recombinant adenoviral vector was injected
into the vitreous humor (posterior segment) of the eye
in anesthetized rats. Control rats were treated in the
same way, but received vehicle alone. 24 hours later
the animals were sacrificed, and the eyes were mounted
30 in Tissue Tek, frozen, sectioned and stained for β -
galactosidase protein. There was clear positive
staining of some of the cells of the choroid, which is
the vascular coat surrounding the posterior part of the

eye. There was no such staining in animals that received vehicle alone.

CLAIMS

1. A method for generating a genetically-engineered *in situ* ocular cell, said method comprising contacting an ocular cell with exogenous nucleic acid under conditions permissive for the uptake of said exogenous nucleic acid into said ocular cell for expression therein.
2. A method according to Claim 1 wherein said exogenous nucleic acid is in the form of a retrovirus.
3. A method according to Claim 1 wherein said exogenous nucleic acid is in the form of an adenovirus.
4. A method according to Claim 1 wherein said exogenous nucleic acid is in the form of a plasmid.
5. A method according to Claim 1 wherein said exogenous nucleic acid is in the form of an adenoassociated virus.
6. A method of treating ocular disease comprising incorporating exogenous nucleic acid into an *in situ* ocular cell under conditions permissive for the uptake of said exogenous nucleic acid, said exogenous nucleic acid encoding a protein associated with said ocular disease.
7. A method of treating ocular disease comprising incorporating exogenous nucleic acid into an *in situ* ocular cell under conditions permissive for the uptake of said exogenous nucleic acid, said exogenous nucleic acid encoding a protein useful in the treatment of said ocular disease.

8. An ocular cell containing exogeneous nucleic acid.

9. A method according to claim 6 or 7 wherein said cell is a corneal endothelium cell.

5 10. A method according to claim 6 or 7 wherein said cell is a corneal epithelial cell.

11. A method according to claim 6 or 7 wherein said cell is a choroid cell.

10 12. A method according to claim 10 wherein said cell is debrided prior to introducing said exogeneous nucleic acid.

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The invention relates to methods of ocular gene therapy.

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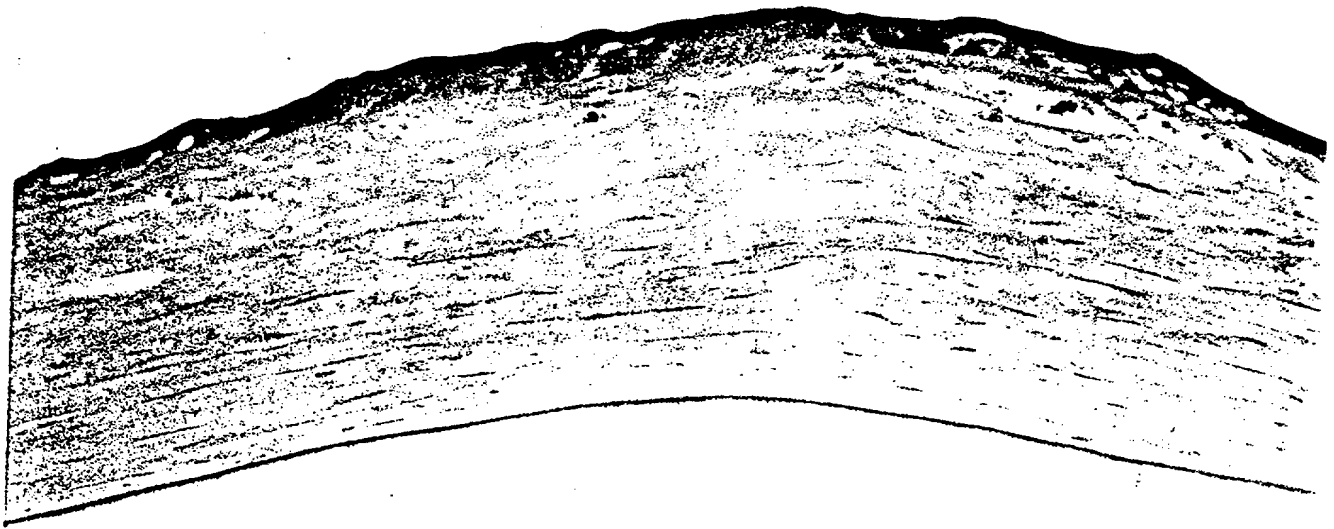


Fig. 1A

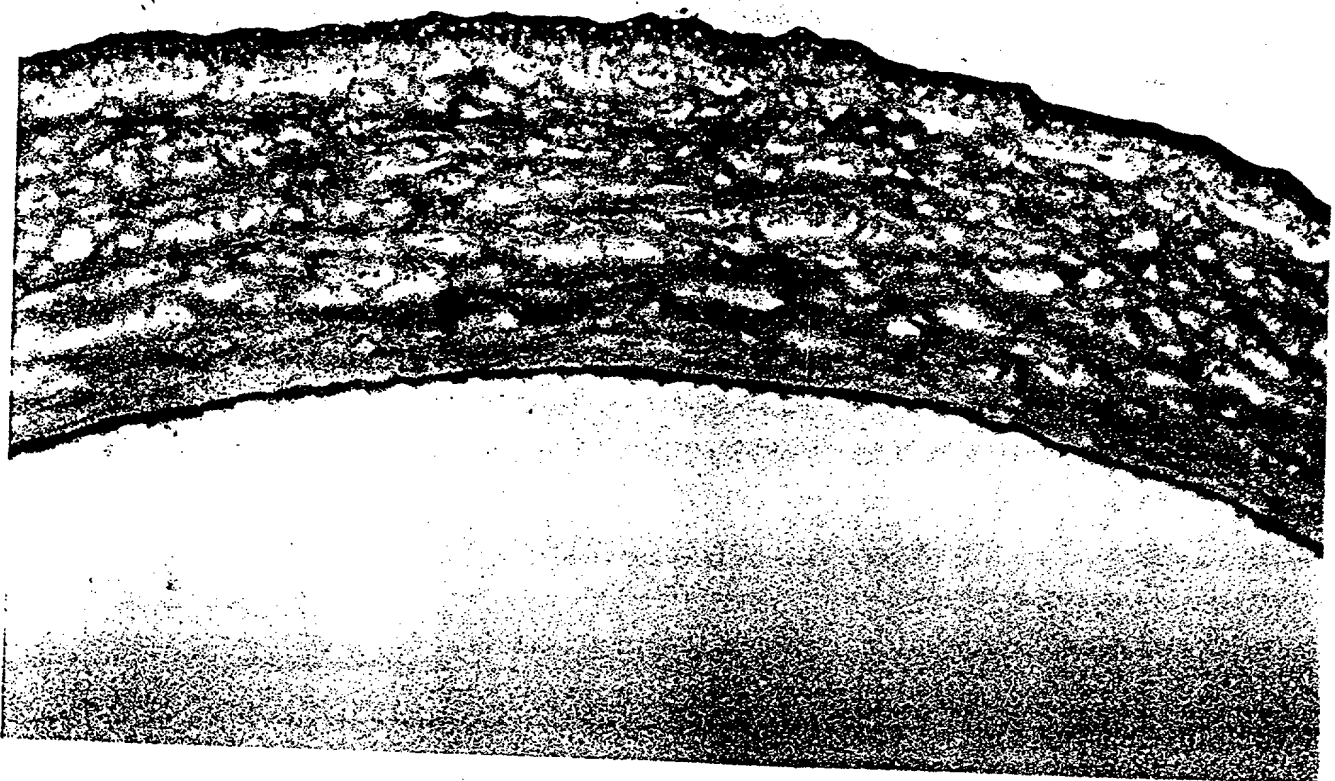


Fig. 1B

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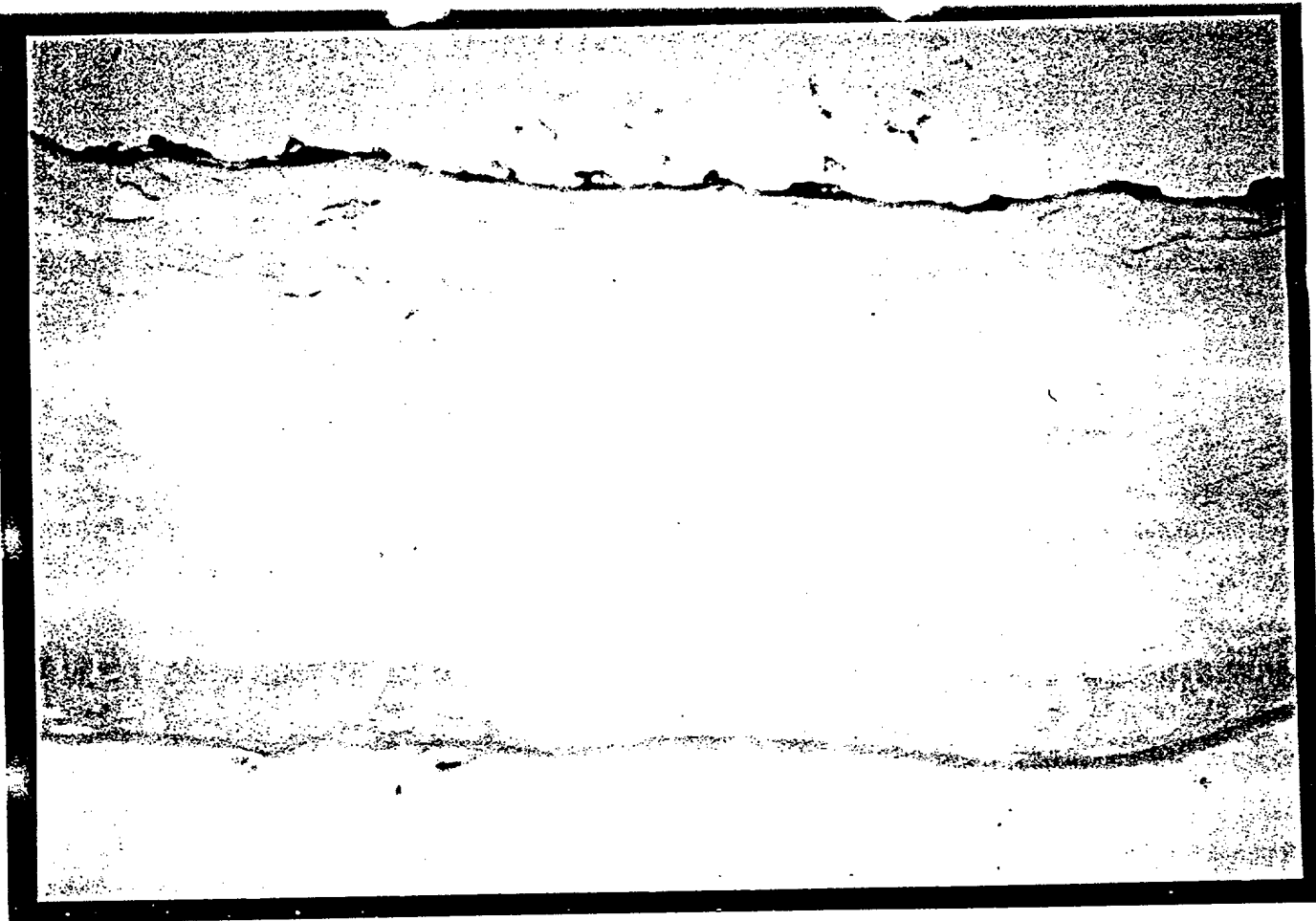


Fig. 2A

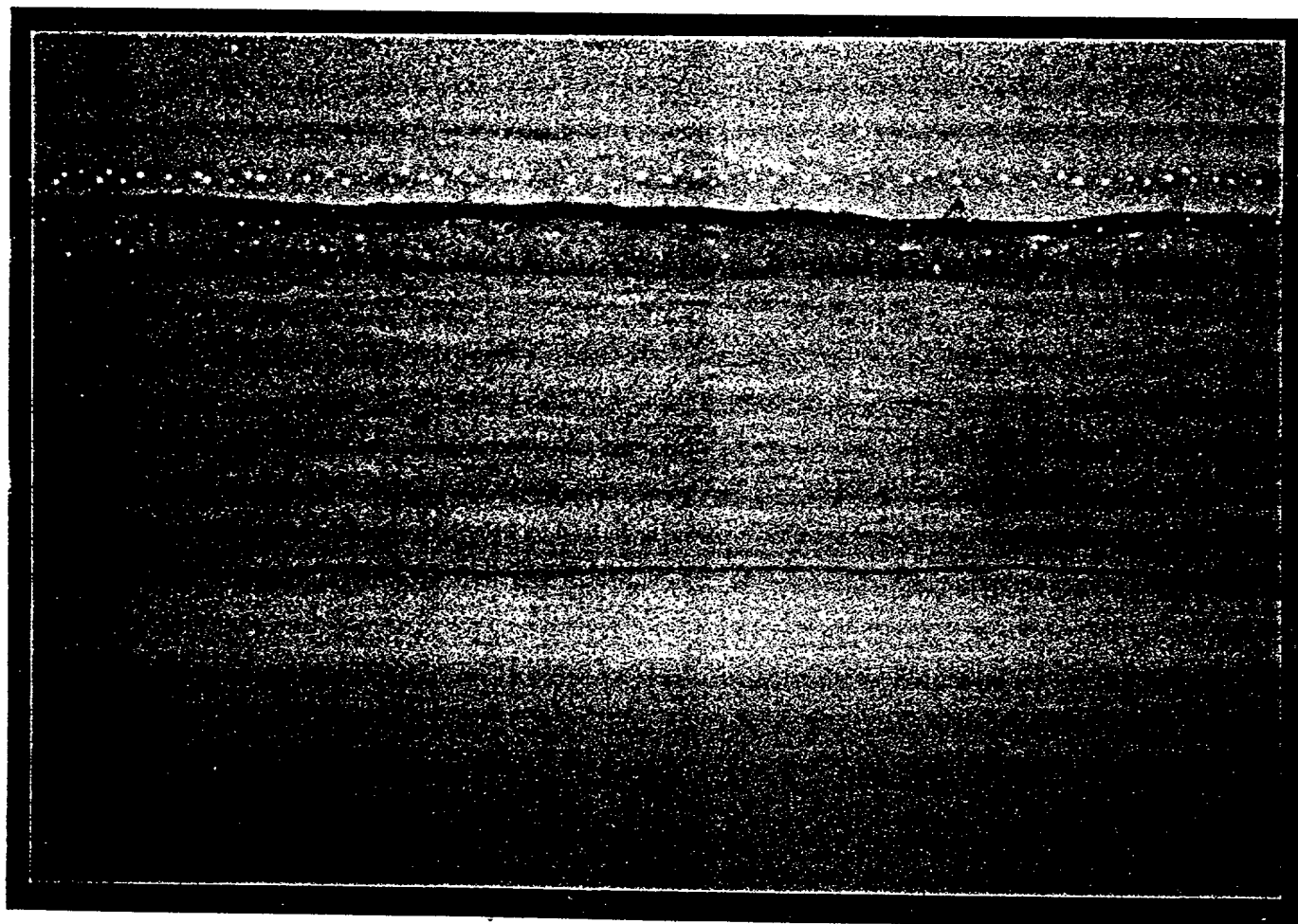


Fig. 2B

DECLARATION FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

OCULAR GENE THERAPY

the specification of which

(check ☐ is attached hereto.
one)

☒ was filed on October 31, 1994 as
Application Serial No. 08/332,566
and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I/We acknowledge the duty to disclose to the Office all information known to me/us to be material to patentability as defined in §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulation, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

_____	_____	_____
(Application Serial No.)	(Filing Date)	(Status)
		(patented, pending, abandoned)
_____	_____	_____
(Application Serial No.)	(Filing Date)	(Status)
		(patented, pending, abandoned)

Direct all telephone calls Walter H. Dreger at (415) 781-1989.

Address all correspondence to:

FLEHR, HOHBACH, TEST,
ALBRITTON & HERBERT
Suite 3400, Four Embarcadero Center
San Francisco, California 94111

File No. A-59553/WHD/RMS

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18, United States Code, §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or
first inventor:

R. Andrew Cuthbertson

Inventor's signature:

Rushinso

Date:

1/11/95

Residence:

San Francisco, California

Citizenship:

Australia

Post Office Address:

559 Douglass Street

San Francisco, CA 94114

~~Full name of second joint
inventor, if any:~~

Inventor's signature:

Date:

Residence:

Citizenship:

Post Office Address:

Full name of third joint
inventor, if any:

Inventor's signature:

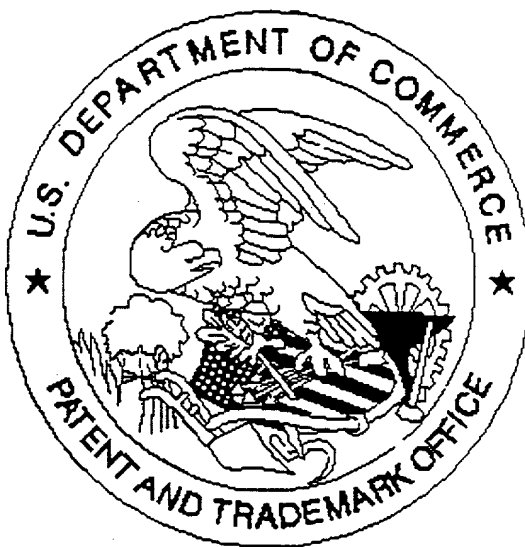
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